

Analysis and Mechanisms of Cyclotrimethylenetrinitramine Ion Formation in Desorption Electrospray Ionization

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The general ion chemistry of the explosive molecule cyclotrimethylenetrinitramine (RDX) was studied with an atmospheric pressure ionization mass spectrometer (API-MS) fitted with a desorption electrospray ionization (DESI) source. Explosive molecule chemistry within trace detection techniques such as ion mobility spectrometry (IMS) is an area of intense interest because of the widespread deployment of IMS-based explosive detectors for counterterrorism efforts. As in IMS, the DESI-MS experiments analyze material that starts in the solid phase and is detected in the gas phase. Using the unique chemical characterization inherent in mass spectrometry, information pertinent to the atmospheric ionization of RDX is obtained in order to help explain the behavior of explosive molecule signatures observed within IMS experiments. Qualitative and quantitative information was obtained over 3 orders of magnitude of deposited mass (nanograms to greater than micrograms). A method was developed to use the relative integrated mass spectral peak intensities of RDX monomer and dimer chloride adducts to determine the amount of explosive present on a surface. The ratio of RDX dimer chloride adduct to monomer chloride adduct ranged from 0.1 for 15 ng to 1.0 for 1.5 μg of deposited explosive. The results are explained in terms of mechanisms reported in the literature for electrospray ionization (ESI), as well as by simple solution dynamics and the interaction chemistry between RDX molecules. On the basis of all available data, the RDX dimer chloride adduct becomes disproportionately favored over the monomer chloride adduct at larger amounts of explosive because of effects related to desorbed droplet charge, solvent declustering, and the strong intermolecular forces between RDX molecules in the solid, liquid, and gas phases. Additionally, considerations for optimization of the DESI-MS process are described in order to increase the practicality for this technique as an explosives detection tool in the public domain.

Trace detection of explosives is an area of global importance in connection with issues of national security. The chemistry of explosive molecules has been studied for decades, but the behaviors of these molecules in the solid, liquid, and gas phases

are still not completely known. Detailed understanding of explosive molecule chemistries in the solid and gas phases is critical to improve the design and efficiency of ion mobility spectrometry (IMS)-based detectors, which are widely deployed for explosives trace detection.

Several new techniques have been recently developed for the analysis of explosive molecules, and many of these utilize atmospheric pressure mass spectrometry. A thorough review of some of the new methods for atmospheric pressure mass spectrometry ionization was recently published.¹ Briefly, these techniques can be distinguished into those related to electrospray ionization (ESI) and those related to atmospheric pressure chemical ionization (APCI). The ESI-related techniques include desorption electrospray ionization (DESI),² desorption sonic spray ionization (DESSI),³ and the laser-based ionization methods such as electrospray laser desorption/ionization (ELDI)⁴ and laser ablation electrospray ionization (LAESI).⁵ The APCI-related techniques include direct analysis in real time (DART),⁶ helium atmospheric pressure glow discharge ionization (HAPGDI),⁷ and plasma-assisted desorption/ionization (PADI).⁸ In general, the atmospheric pressure-based ionization methods allow for the surface analysis of materials *without* sample preparation. This simplification of analysis protocols has particular relevance in the areas of biological tissue analysis, explosives detection, and forensic analysis.

In terms of surface analysis techniques, DESI is the liquid-based, atmospheric pressure analogue to secondary ion mass spectrometry (SIMS), which has been successfully used for decades in the surface analysis of inorganic, organic, and more recently, biological materials. Instead of the energetic ions used in ultrahigh vacuum conditions in SIMS (and the related fast atom

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bombardment—FAB), the DESI desorption event occurs at ambient pressure and generally consists of polar solutions or solvents sprayed through a silica capillary. Droplet velocities are generated via a coaxial flow of pressurized gas as well as a potential applied to the spray emitter. The droplets impact the target surface, and material is desorbed within smaller droplets that traverse to the vacuum interface of a mass spectrometer. Fundamentally, it would be expected that the mechanisms of DESI are some combination of processes involved in the ionization events in SIMS/FAB and ESI.

For the analysis of explosives and related materials, DESI has proven to be efficient at desorbing ions from a variety of surfaces and producing mass spectra that are unique to each explosive molecule.^{9–15} The molecules cyclotrimethylenetrinitramine (RDX), cyclotetramethylenetetranitramine (HMX), tetranitropentaerythritol (PETN), trinitrotoluene (TNT), and triacetone triperoxide (TATP) have been successfully analyzed using DESI on a range of surfaces, including human skin, paper, and luggage materials.^{10–15} Since the general approach in the above references has been to achieve the best detection limits in the mode of “trace detection” of explosives, the amount of surface material rarely exceeded tens of nanograms. On the basis of previous DESI laboratory results involving explosives,^{9–15} it is unknown whether this new form of ionization can be of quantitative use *above* trace detection levels of RDX that are into the microgram range of solid material. A recent study determined that the amount of RDX present in human fingerprints after handling the plastic-bonded explosive C-4 can exceed microgram levels of explosive material.¹⁶

RDX is a practical test molecule for studying the fundamentals of DESI-MS for explosives analysis. The gas-phase chemistry is interesting, as it has a propensity for reacting with dopant gases to form a variety of adducts, and it is a target for trace detection globally due to its presence in plastic-bonded explosives such as C-4.^{13,16} Additionally, DESI-MS can become a practical tool for testing the chemistry of current and future IMS parameters, dopant gases, and possible gas-phase reactions, since IMS-based instruments often detect explosive molecule adducts. The parameters used here for the DESI-MS process are unique to this study, in that we have used amounts of deposited explosive material appropriate for fingerprint detection of RDX¹⁶ and have focused on understanding the ion processes inherent in both DESI-MS and IMS. This study uses the first commercially available DESI source designed for an Applied Biosystems instrument and delves into several of the issues that need to be resolved to carry out such research on a 4000 QTrap triple quadrupole, linear ion trap mass spectrometer. (Certain commercial equipment, instruments, or materials are identified to adequately specify the experimental

procedure. Such identification does not imply recommendation nor endorsement of the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.) Specifically, the results are discussed in terms of previously reported mechanisms of ESI, with specific differences of the DESI technique highlighted.

EXPERIMENTAL SECTION

Sample Preparation. Cyclotrimethylenetrinitramine was obtained from Restek (Bellefonte, PA) at 1 mg/mL concentration in acetonitrile and diluted to the appropriate concentrations for this study with 1:1 methanol/water. HPLC-grade methanol (Mallinckrodt Baker, Phillipsburg, NJ) and HPLC-grade Chromasolv water (Sigma-Aldrich, St. Louis, MO) were previously combined to form a miscible solution. Sodium chloride (Sigma-Aldrich, St. Louis, MO) was dissolved into the HPLC-grade water as needed. Aliquots of 3 μ L of each diluted RDX solution were deposited onto glass slide well plates, where each well plate consisted of a heavily hydrophobic coating (purchased through ProSolia, Inc. but manufactured by Cel-Line, Portsmouth, NH). The explosive material was deposited in a circular area so that the size of the aliquot before evaporation was comparable to the DESI beam spray size. Deposited solutions were allowed to dry in air for approximately 15 min before being directly analyzed with the DESI microdroplet beam.

Instrumentation. *DESI.* A ProSolia (Indianapolis, IN) OmniSpray ion source made specifically for the Applied Biosystems/MDS Sciex 4000 QTrap mass spectrometer was affixed to the MS using an Applied Biosystems/MDS Sciex nanospray interface and an extension tube inlet provided with the ion source. The solution composition used for generating the microdroplet beam was obtained from work done elsewhere.¹⁴ Briefly, a 1:1 methanol/water solution with 1 mmol/L sodium chloride salt was directed at the surfaces of interest. A syringe pump from kd Scientific (Holliston, MA) was used to generate and maintain the solvent flow through the DESI system at 4 μ L/min from a Hamilton (Reno, NV) 1 mL gastight syringe. The coaxial carrier gas was set to 100 psig of nitrogen (N_2) from a house bleed-off of a liquid nitrogen dewar. The DESI spray voltage was operated at -1 kV unless otherwise noted and is set via the Applied Biosystems Analyst instrument software for potentials of the same polarity as the detected ions. The -1 kV potential was determined by maximizing total ion signal response as a function of tip voltage, spray conditions, and the various possible DESI source geometries. For opposite-polarity potentials applied to the DESI tip, a model 205B-05R, 0–5 kV adjustable external power supply (previously Bertan Associates, Inc., now Spellman, Hauppauge, NY) was employed. The DESI source was directed at the surface at an incident angle of 55° with respect to the sample plane with the following geometries: tip to surface—approximately 2 mm, surface to tube inlet—approximately 2 mm, and tip to tube inlet—approximately 3 mm. The stage z -axis was set to 20.70 mm, and the y -axis was set to 25.23 mm.

Mass Spectrometer. A 4000 QTrap Triple Quadrupole LC–MS system (Applied Biosystems/MDS Sciex, Foster City, CA/Toronto, Canada) was fitted with the optional nanospray interface. Despite the instrument’s ability to perform advanced tandem mass

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Table 1. Parameters for the 4000 QTrap Fitted with a DESI Ion Source^a

pressures (psi)	temperature	voltages (V)	mass spectra
curtain gas: 20.0	interface heater: 100 °C	declustering potential: -70	30 s Q1 scans
ion source gas 1: 12.0		entrance potential: -10	40 summed scans
ion source gas 2: 0.0		ion energy: -0.5	<i>m/z</i> 40–750
operating vacuum: 0.8–1.0 × 10 ⁻⁵ Torr		multiplier: 2200	0.1 amu step size

^a Certain commercial equipment, instruments, or materials are identified to adequately specify the experimental procedure. Such identification does not imply recommendation nor endorsement of the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

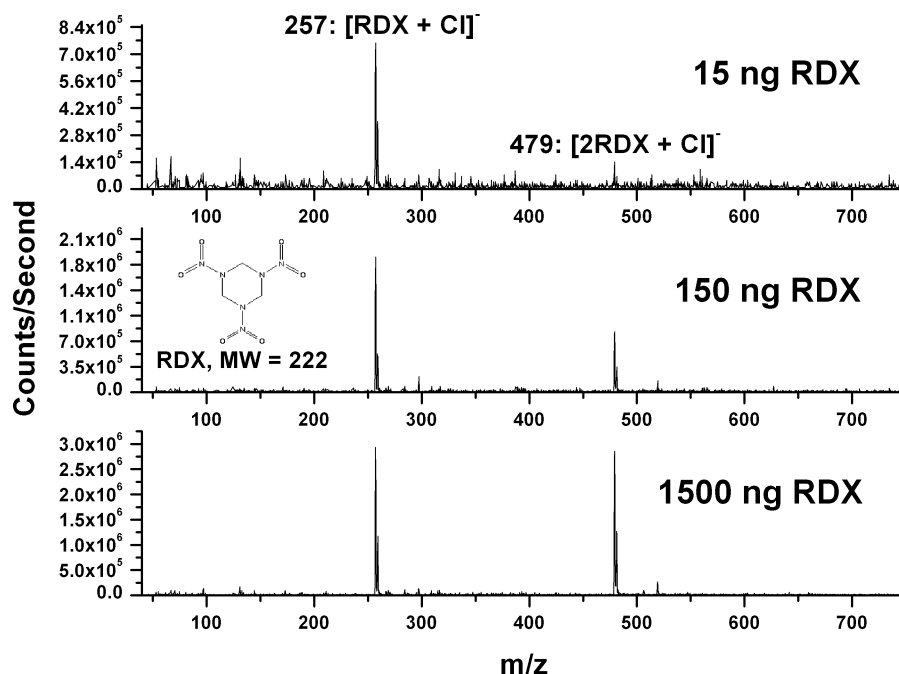


Figure 1. Background-subtracted DESI-MS spectra for deposited RDX amounts of 15 ng, 150 ng, and 1.5 μg , showing the increase in dimer to monomer peak ratio with increasing deposited material. A structure of cyclotrimethylenetrinitramine (RDX) is provided.

spectrometry modes such as MS/MS, MS/MS/MS, multiple reaction monitoring, neutral loss scans, and enhanced resolution scans, the instrument was primarily used in a mode where the first quadrupole is scanned over a mass range of interest, with the second and third quads being inactive. The targeted mass spectra are well-documented in the literature, and although MS/MS was used to ultimately verify the identity of the observed mass spectral signals for RDX, repeated use of tandem mass spectrometry was not necessary for the scope of this study. Table 1 summarizes the key 4000 QTrap parameters used for this work.

RESULTS AND DISCUSSION

RDX Signal Response versus Amount of Deposited Material. Negative-ion DESI-MS spectra of 15 ng, 150 ng, and 1.5 μg of deposited RDX are produced by mass analysis with the 4000 QTrap and shown in Figure 1, along with an image of the RDX molecule. As reported previously, the addition of chloride anions via NaCl into the solvent spray results in mass spectra that demonstrate strong chloride adduct formation.^{10,11,13,14} The first set of peaks representing RDX in the spectra contain the adduct of the RDX monomer plus chloride at *m/z* 257 and 259. Also visible in the mass spectra are the RDX dimer plus chloride adducts at *m/z* 479 and 481. Aspects of the DESI-MS spectra that are advantageous for explosives analysis include (1) RDX being easily

identified because of the lack of molecular fragmentation producing a simple and clean mass spectrum, and (2) similar to dopant chemistry in IMS experiments, the chloride attachment enhances the overall RDX-specific signal response for the targeted masses because of the strong preference of RDX to form adducts with chloride ions instead of solvent-related clusters. The spectra in Figure 1 and much of the other data presented in this manuscript are background-subtracted to more easily discern the explosive-specific peaks at low concentrations. (The background subtraction scheme is included in the Supporting Information.)

Comparisons of DESI-MS spectra ranging from 15 ng to 1.5 μg of deposited RDX can also be made from Figure 1. Aside from the expected increase in signal response with increasing amounts of solid RDX on the well plates, special attention is given to the behavior of the RDX dimer chloride adduct ion at *m/z* 479 relative to the RDX monomer chloride adduct ion at *m/z* 257. When 15 ng of RDX is probed with the DESI beam, the mass spectrum is dominated by the monomer adduct, with a small component of the dimer adduct visible just above the baseline. As the amount of deposited RDX is increased 10-fold to 150 ng, the height of the dimer adduct increases substantially to about half the height of the monomer adduct. When the deposited amount of RDX is increased another 10-fold to 1.5 μg , the dimer adduct increases further to approximately equal signal height to the monomer

adduct of RDX. Both monomer and dimer RDX–chloride adducts are reported in the recent literature, but little attention is directed at understanding the nature of dimer formation in DESI-MS. In actuality, the relative amounts of dimer and monomer adducts are rather inconsistent in published DESI mass spectra^{10,11,13,14} and ESI mass spectra.¹⁷ It is known in ESI that subtle changes in solution components and impurities, as well as overall RDX concentration, can dramatically change the abundances and chemical identities of RDX-specific adduct ions in the mass spectra, making quantification difficult.¹⁷ However, in the DESI droplets, little explanation has been provided as to the physicochemical processes that drive ion formation for the dimer molecules, nor has attention been paid specifically to the dimer adduct behavior. Other previously reported features in the Figure 1 mass spectra, and visible in the 150 ng and 1.5 μg samples, are solvent adduct peaks at m/z 297 and 519 of an RDX monomer and dimer, respectively, attached to a m/z 75 adduct from the specific methanol/water beam composition.¹¹ Of note, a trimer RDX species is not observed in the DESI mass spectra reported here, which is consistent with recent ESI-MS data of a similar system.¹⁸

Previous attempts at quantification with DESI-MS of explosives has been limited, with reports of sodiated TATP exhibiting a linear signal response with respect to solid analyzed material in the approximate range of 1 ng to 5 μg .¹⁵ TNT molecular ions have been quantified with DESI over 3 orders of magnitude in a similar range to the TATP data.¹¹ Quantification of RDX has been attempted between the range of one to tens of picograms with positive-ion adducts, where “saturation of the response” was observed.¹¹ The range of quantifiable RDX was 0.5–10 ng for negative chloride adduct ions,¹¹ but this data still represents a small quantifiable linear dynamic range compared to other explosives. It is conceivable that quantification has been difficult with the explosives that dimerize well, such as RDX, HMX, and PETN, because the signal response can be split between two ionization channels (one for monomer and another related, but separate, channel for the dimer). We have observed inconsistent signal response of the raw integral counts of RDX-specific mass spectral peaks with DESI. Therefore, we have instead chosen to use a different approach at quantification by using the ratio of integral counts of the RDX dimer adduct to the RDX monomer adduct as a function of increasing deposited mass. The ratio of RDX dimer adduct to monomer adduct signal response versus deposited material is plotted in Figure 2. Each point represents the average ratio of dimer to monomer for at least seven replicate experiments for a given amount of deposited RDX. The previously reported linear range of quantifiable RDX using the monomer signal response¹¹ overlaps with the range at the beginning of the curve in Figure 2. If the previous data were superimposed on this plot, we predict that the linearity would end somewhere between the first and second data points. After that range, a deviation from linearity of response as the amount of RDX increases into the microgram range is observed in Figure 2. [A previous set of data that was less comprehensive in the overall range of deposited RDX produced a similar curve shape (data not shown), and a more

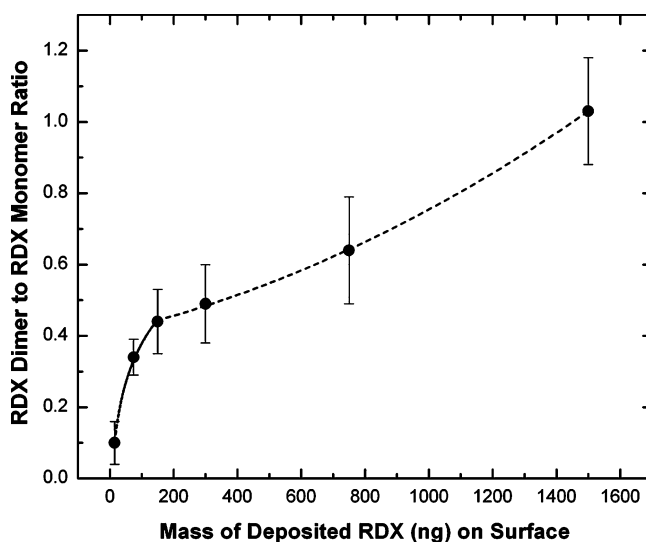


Figure 2. Plot of ratio of RDX dimer chloride adduct at m/z 479 to RDX monomer chloride adduct ion at m/z 257 versus mass of deposited material. Dotted lines are provided in order to display a point of overlap between two different curve shapes. Each point is the average of between seven and eighteen experiments, with the error bars representing the standard uncertainties at each point.

detailed analysis produced the results displayed in Figure 2.] The fit of the dotted lines reveals a crossover region of signal response between two different and overlapping curve shapes, which will be explained further in subsequent sections.

Proposed Mechanisms of RDX Monomer and Dimer Adduct Ion Formation. In general, it is expected that, once material is desorbed from the surface with the DESI beam, droplet physics will be very similar to those observed in ESI. There should also be some basic solvent dynamics present in the DESI desorption event because of the large amount of liquid phase directed at the sample in comparison to the amount of analyte. In the lower-concentration region, if the DESI spray dynamics stay constant, an increase in surface analyte should theoretically move the analysis from a regime of an infinitely dilute solute within the DESI droplet pool to one where the increasing amount of analyte generates a larger signal response. A critical concentration can be reached in which there is no further increase in signal response either because the desorbed droplets saturate with analyte, or the droplet charge is limited, or both—but each limiting factor would influence the ionization pathways of monomer and dimer adduct ions. For the higher-concentration region of the plot in Figure 2, in which the dimer to monomer ratio continues to increase with increasing amounts of deposited RDX, solute/solvent dynamics may be dominating the curve shape. For example, interaction forces between molecules in solution generally fall off with distance as $1/r^2$ for this type of system,¹⁸ where r represents the distance between molecules. In fact, a simple $1/r^2$ plot is similar to the higher-concentration region in Figure 2, albeit slightly sharper in signal rise than the RDX data indicates. As the desorbed droplets in DESI, and more traditional ESI, travel through the vacuum interface to the mass spectrometer, a large degree of solvent declustering occurs. During this time, the available charge density on the droplet periphery continues to increase as droplet size decreases, or until Coulombic repulsions split the droplets into smaller droplets. Correspondingly, the analyte concentration within the droplets continually increases

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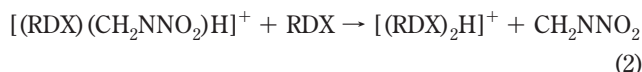
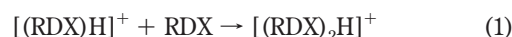
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during solvent declustering toward a saturated solution, which results in a net decrease of the interaction distances between molecules. With increasing analyte concentrations in the desorbed DESI droplets, the molecules will enter a regime where the magnitude of the van der Waals forces between molecules increases, which increases the probability of molecules sticking together as the droplets decrease in size. These smaller interaction distances would favor the eventual production of intact RDX dimer adducts over monomer adducts disproportionately as the concentration increases, similar to what is observed in the higher-concentration region of the curve in Figure 2. The less sharp increase of the polynomial curve fit with respect to a typical $1/r^2$ relationship can be a byproduct of the $1/r^2$ -style plot mixing with the asymptotic signal response relationship exhibited in the lower-concentration region. The crossover region of signal response curve shapes in Figure 2 denotes the area where a theoretical asymptote and beginning of the $1/r^2$ relationship both overlap. In order to determine if these ideas are plausible, we have evaluated the published work of related systems, with the discussion of the proposed mechanisms in the next few sections.

Proposed Mechanisms in Relation to Desorption Effects and Droplet Charge. Recent computational fluid dynamics simulations (CFD) of the DESI impact event provide insight into the mechanisms present during the desorption process.¹⁹ The authors found that the DESI event consists of a “two-step droplet pickup mechanism”.¹⁹ Specifically, it was suggested that a pool of the DESI spray develops on the surface, and a subsequent droplet impact desorbs material from the pool, of which the desorbed droplets of certain velocity and angular distribution enter the mass spectrometer.¹⁹ This mechanism requires the material present on the surface to be incorporated into the formed droplet pool *before* being desorbed into the mass spectrometer. This information takes the molecular distribution of analyte on the surface of interest and converts any potential solid-phase-based mechanisms to those in the solution phase. Therefore, the simple notion of linear signal increase as the analyte concentration increases from infinitely dilute to slightly higher values is still expected. In the higher-concentration region, as the RDX approaches microgram levels of surface material, the droplet pool may approach a saturated solution of analyte. In both the lower- and higher-concentration regions of Figure 2, the declustering droplets naturally approach a saturated solution once desorbed, but for the higher-concentration region, the aforementioned interaction distances may not just be a factor in the sputtered droplets, but in the droplet pool as well. The possibility exists that RDX dimer adducts may not just be forming in the declustering droplets but may actually be *desorbed* as intact entities from the solution pool just above the analyte surface. As for the overlap region of the curve in Figure 2, beyond a simple combination occurring of the mechanisms listed above, there is a possibility of a competition among monomer and dimer units for droplet charge. An equilibrium model for competition of the excess droplet charge between RDX monomers and dimers was recently published for liquid chromatography (LC)–ESI-MS.¹⁸ This model assumed that a partitioning of the monomer and dimer RDX molecules into the charged perimeter of the ESI droplets must

occur. It was then reasoned that the dimer would more easily partition to the droplet periphery because of its increased hydrophobicity compared to the monomer—and its propensity to want to get as far as possible from the polar solvent molecules in the core of the ESI droplets.¹⁸ This mechanism may be contributing to the observations in Figure 2, where at higher concentrations, the ratio of RDX dimer adduct to monomer adduct appears to continue increasing with amount of surface material.

Proposed Mechanisms in Relation to Adduct Formation. The impact of competition for available Cl^- ions throughout the entire concentration region cannot be overlooked since the anions are needed for the RDX adducts. The question then becomes, do the chloride ions attach to monomers and dimers of RDX separately, or does a neutral RDX molecule attach to an already existing RDX–Cl monomer adduct? Previously, protonated RDX monomer and dimer molecules were studied by Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) in order to determine the mechanisms of dimer formation.²⁰ The particularly abundant protonated RDX dimer was found to *not* be primarily formed by the direct interaction of a protonated RDX monomer and a neutral RDX, as illustrated in reaction 1.²⁰ Instead, it was found through a series of gas-phase reactions in the FTICR-MS experiments that the scheme in reaction 2 was dominant.²⁰



The second reaction requires some of the original RDX molecules to be fragmented into methylenenitramine groups before attaching to RDX monomer molecules. These in turn react with RDX neutrals to produce the protonated RDX dimer and to liberate a neutral methylenenitramine unit (which can react with more free RDX).²⁰ However, it cannot be assumed that the protonated form of the RDX monomer and dimer molecules would behave in the same manner as RDX adducts with Cl^- ions. Additionally, the reaction scheme above was the result of the RDX molecules being subjected to 70 eV of electron impact ionization (EI),²⁰ which would appreciably fragment the RDX molecules. As was already mentioned, the DESI-MS spectra reported here show no fragmentation of the RDX molecular structure, thereby eliminating the type of mechanism of dimer formation illustrated in reaction 2. In other published work, nuclear quadrupole resonance spectroscopy (NQR) was used to study the relationship of RDX dimers in the solid phase with thermal decomposition.²¹ Specifically, it was found that RDX–RDX pairs do exist in the solid phase as the primary means of structural organization and that interactions between oxygen and nitrogen atoms, as well as nitrogen to nitrogen interactions, keep the pairs together.²¹ The authors stated that two of the RDX nitro groups experience stronger intermolecular forces with another RDX molecule than the remaining nitro group, due to the orientations of the RDX molecules in a dimer pair.²¹ By having one relatively

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free nitro group in each RDX–RDX pair, it was argued by the authors that the molecules were still free to rotate somewhat while still remaining a dimer and that this plays a role in the speed of RDX decomposition at its melting point.²¹ We propose that having nitro groups in RDX pairs available for interactions with other species could allow for the attachment of a chloride ion as seen in the DESI-MS spectra. In ref 21, the authors mention that the NO₂ group nitrogen atoms have positive partial charges relative to many of the other atoms in the RDX–RDX dimer pairs. This assertion, along with two total nitro groups experiencing little intermolecular forces in the RDX dimer units, may provide “pockets” for an electronegative chloride ion to settle. If prior work is related to the present study, it seems that it is more likely that the RDX dimer chloride adducts form by association of a chloride ion with an already present RDX dimer molecule as proposed in reaction 3, instead of an RDX monomer chloride adduct attaching to a second RDX molecule, as proposed in reaction 4.



If the pathway in reaction 3 is true, the forces holding RDX molecules together would have to be strong enough to survive the desorption process in DESI-MS, which is evident by the strong dimer signals in the mass spectra. Recent computer simulations that modeled the interactions between solid-phase RDX molecules showed van der Waals forces, and more specifically, electrostatic forces and dispersion forces, were responsible for keeping RDX dimer molecules intact.²² The authors found that dispersion forces were considerably more important than electrostatic forces in keeping the tightly bound RDX molecules together and concluded that an absence of dispersion forces altogether resulted in either weak bonding or no bonding of the RDX molecules.²²

The aforementioned LC–ESI-MS study in which excess chloride ions were added postcolumn to separated RDX molecules showed that, with increased concentration of RDX, the monomer chloride adduct reached a signal rollover, but the dimer chloride adduct response was “concave-up” at low concentrations below an estimated 10⁻⁵ mol/L, before changing shape to reach its own signal rollover.¹⁸ To determine if this effect is happening in the DESI-MS experiments reported herein, Figure 3 displays a plot of the separated RDX monomer and dimer chloride adducts, normalized to the baseline solvent ion at *m/z* 131, which was found to remain constant in signal regardless of analyte concentration. (Note that the 750 ng RDX data points are missing in Figure 3. The DESI source geometries were different for that experiment, which affected the amount of solvent molecules entering the MS interface.) The shape of the monomer signal response versus deposited material directly mimics the signal rollover curves reported for ESI-MS in the literature. The ESI-MS work concluded that the dimer adduct signal rollover was consistent with a “stepwise addition” of the observed RDX chloride dimer, as proposed in reaction 4 above, and that the monomer signal experienced a rollover itself because of the onset of increased

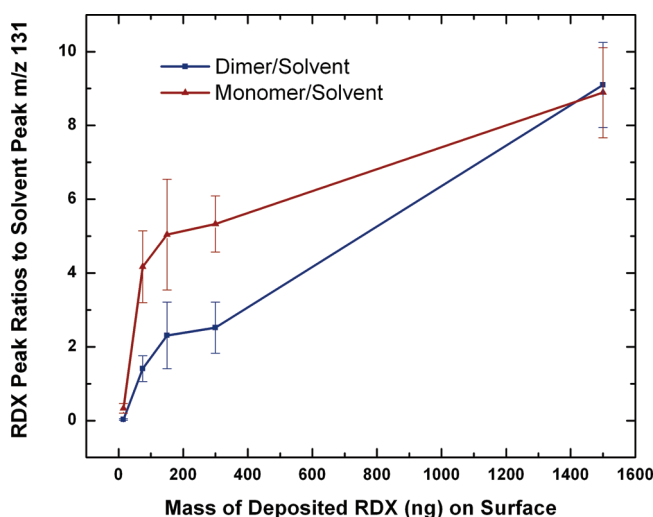


Figure 3. Plot of normalized signals of RDX dimer chloride adduct ion and RDX monomer chloride adduct ion to solvent background signal at *m/z* 131.

dimer formation.¹⁸ Their estimated structure involved chloride attachment to electrophilic hydrogens via ion–dipole interactions only, whereas the RDX molecule resides in a chair conformation.¹⁸ The argument was made that via the same interactions, a second RDX molecule in the same orientation as the first can be stabilized by the other side of the chloride ion, 180° away from the first RDX molecule.¹⁸ We believe that the precise steric specificity of the RDX molecules required for reaction 4 to work seems a bit of a stretch to dominate all possible reaction pathways en route to RDX dimer adduct formation. This is at least due in part to the randomness of molecular conformations possible in the solution and gas phases in the DESI experiments leading to multiple dimer conformation possibilities. Additionally, the dimer chloride adduct plot in Figure 3 *does not* rollover like the published ESI data, which suggests that the “stepwise addition” of RDX + Cl + RDX as in reaction 4 is not the dominant pathway for DESI-MS. We conclude from a summation of previously published work that the multiplicity of forces of varying strengths seem to be sufficient enough to allow RDX molecules to easily associate into dimer molecules in the solid, gas, and solution phases. If the concentration of RDX molecules in the solution-phase droplet pool and/or the solution component of desorbed DESI droplets is high enough, intact dimer units can be expected. Thus, the notion that a chloride attaches to an already existing RDX dimer seems reasonable and can be considered an appreciable, if not dominant, mechanism of RDX dimer adduct formation in these experiments.

Proposed Mechanisms Related to Electrospray Ionization.

A focus on other ESI literature can provide more insight into the mechanisms responsible for some of the observations in Figures 2 and 3. Two publications concluded that signal rollover to an asymptotic value similar to that seen in Figure 3 and the ESI work in ref 18 can be expected for most analytes when using ESI-MS.^{23,24} An “analyte saturation” and resulting signal rollover were commonly seen above solution concentrations of 1 × 10⁻⁵ mol/L for singly charged ions, whereas low-concentration regions of the response curves were linear.^{23,24} One reasoning for the observa-

(22) Podeszwa, R.; Bukowski, R.; Rice, B. M.; Szalewicz, K. *Phys. Chem. Chem. Phys.* **2007**, *9*, 5561–5569.

(23) Zook, D. R.; Bruins, A. P. *Int. J. Mass Spectrom Ion Processes* **1997**, *162*, 129–147.

(24) Enke, C. G. *Anal. Chem.* **1997**, *69*, 4885–4893.

tion pointed to "ion evaporation" models and direct correlations between the ion formation efficiency in the ESI droplets and the amount of excess charge on the droplets, similar to the work discussed earlier by Sigman et al.¹⁸ Once the concentration of analyte molecules in a droplet exceeds the available charge on the perimeter of the declustering droplet, it does not matter if the concentration increases further because those ions will have no ionization pathway and will thus traverse through the vacuum interface as neutrals (and hence, will not be detected as ions). These observations change somewhat when moving from singly charged monomer ions to singly charged dimer molecular ions.²³ It was found in the ESI-MS data that, as analyte concentration increased, the dimer molecular ion signals reached signal rollovers at concentrations 1–2 orders of magnitude *above* the same point for the monomer ions. An estimate of the solution-based concentration of the RDX can be calculated to determine if the plot makes sense within the 1×10^{-5} mol/L signal rollover regime mentioned in the literature. We will assume from the CFD simulations in ref 19 that if detected material comes from droplets that have been desorbed from a droplet pool above the analyte surface, then all surface material must be solvated into the pool in order to be eventually detected. For the 30 s of acquisition time that the spray is hitting the targeted material, at a rate of 4 μ L/min, the amount of liquid that the surface comes in contact with can be no more than 2 μ L of the methanol/water and chloride spray solution. For the range of 15 ng to 1.5 μ g in this experiment, the corresponding minimum concentrations range from approximately 4×10^{-5} to 4×10^{-3} mol/L. Reference 23 does point out that some monomer molecules can experience signal rollover at a concentration as high as 10^{-3} mol/L, and to some extent this value will depend on the efficiency of ion transfer through the vacuum interface.²³ However, the response of the dimer curve is different here and closely mimics the ratio plot in Figure 2. This indicates that the dimer response is driving the shape of the dimer to monomer ratio plot for reasons that are not completely related to what has been seen in ESI-MS data. It is likely that the rise in dimer signal response beginning at the observed inception of monomer signal rollover is due to a combination of (1) the ion/molecule dynamics in the DESI droplets mentioned earlier where interaction distances are quickly shrinking as the droplet experiences solvent declustering, (2) the already strong association for RDX molecules to form dimer adducts once the interaction distances in the droplets decrease, (3) the available DESI droplet charge preferentially going to molecules with larger cross sections as the droplet rapidly decreases in size, and (4) the competition for available Cl^- ions from monomer and dimer RDX molecules. One of the mechanisms regarding competition for the droplet charge among analytes in the droplet solution involves the ability of one ion over another to break out of the "solution phase" of the droplet to enter the highly charged perimeter of the droplet,^{18,23} but there is no substantial evidence in this work to support or refute that potential mechanism. The minimum concentration of RDX molecules in the DESI pool, as calculated above, can be used to discuss the competition for Cl^- ions between monomer and dimer RDX molecules. It is extremely likely that those concentrations promote a solution environment where Cl^- ions are in excess for nanogram levels of deposited RDX. Any

monomer and dimer RDX molecules present in solution can attach to a chloride in order to form the detected adducts. However, at microgram levels of deposited RDX, the explosive molecules may be in excess. On the basis of discussions thus far, it is very likely that, at high concentrations of RDX in the DESI pool *and/or* the desorbed droplets, a significant amount of RDX dimer units will be formed. The apparently high stability of the dimer molecules in terms of interactive forces, as well as the larger cross-sectional areas of the dimers compared to monomer molecules, would favor a situation where the dimer molecules can compete more for free Cl^- ions than the monomer molecules.

Effect of RDX Monomer and Dimer Adduct Ion Signal Response versus Acquisition Time. In Figure 4, DESI-MS spectra obtained for 30 s of acquisition time are presented in the case where the DESI spray is constantly impacting 300 ng of initially deposited RDX. The first observation to note is that even after nearly 10 min of constant spray solution (a total of 38 μ L delivered to the target area), that RDX-specific mass spectral peaks are still detectable. (This is important for any potential experimenter that is checking for the presence of explosives on a surface with the DESI-MS technique. For certain analyte targets, complete changes in instrumental parameters can be accomplished while still probing the same sample area.) The other main observation of the mass spectra in Figure 4 centers on the decrease of the RDX dimer chloride adduct relative to the monomer chloride adduct as material is removed from the surface. A plot of the RDX dimer/monomer adduct ratio is shown in Figure 5, and it confirms the decrease in the ratio with increasing acquisition time. This is supported by the previously described mechanisms based on RDX concentration in the droplet pool and subsequently desorbed droplets. As the continual DESI spray strikes the surface, large droplets can displace material from the main droplet pool, while more spray solution continually strikes the droplet pool—both of which decrease the amount of total RDX available for mass spectral analysis via desorption. Less RDX molecules mean less possibility for RDX–RDX interactions to form dimers in either the droplet pool or in the declustering droplets en route to the mass spectrometer.

Effect of RDX Monomer and Dimer Adduct Ion Signal Response versus DESI Tip Voltage. The effect of available droplet charge for RDX ionization can be studied by changing the voltage on the DESI spray tip. From a practical standpoint of potentially using DESI-MS as a means of explosives detection in the public domain, it would be desirable to keep the tip voltage to a minimum, if any at all—in sharp contrast to the DESI-MS literature where explosives are detected with somewhere between 3 and 7 kV on the spray emitter.^{10–15} Figure 6 displays two mass spectra representing the range of RDX-specific signal intensities attainable when *zero* voltage is applied to the DESI tip. The top spectrum represents the baseline level of signal to be expected under zero-voltage conditions, and the bottom spectrum represents the best possible spectrum to be expected under zero-voltage conditions. In both cases where 300 ng of RDX is deposited, RDX monomer and dimer chloride adducts are visible, and although the top mass spectrum has low overall signal, the signal-to-background ratio is still large enough for easy RDX-specific peak identification. The only driving force for the spray to reach the

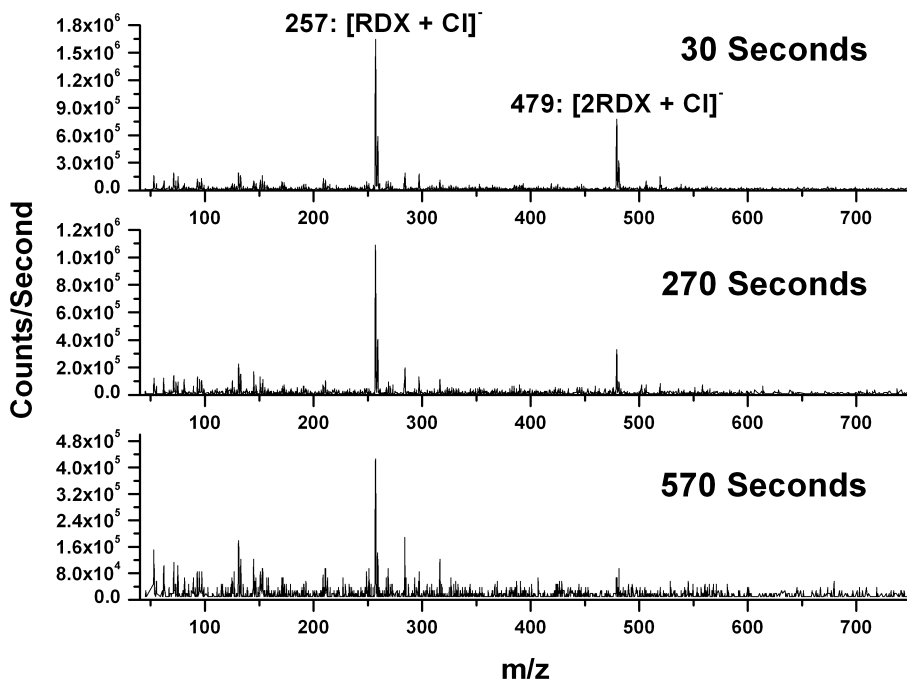


Figure 4. DESI-MS spectra at different time intervals for 300 ng of deposited RDX from a 100 $\mu\text{g/mL}$ solution in 1:1 methanol/water, showing the decrease in dimer to monomer peak ratio as material is removed from the surface.

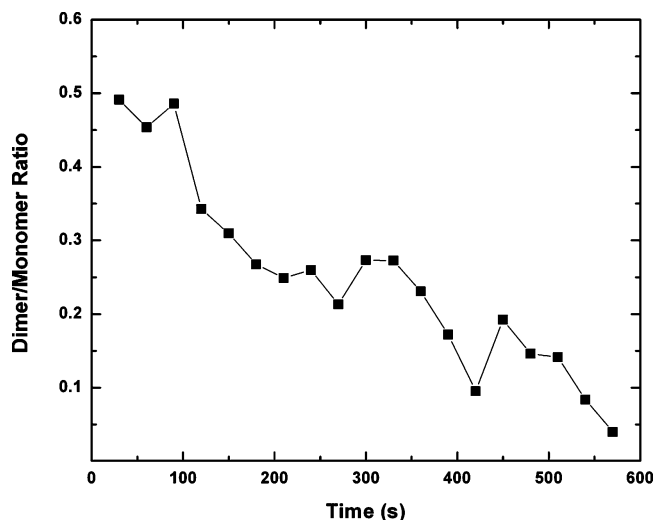


Figure 5. Plot of ratio of RDX dimer chloride adduct ion at m/z 479 to RDX monomer chloride adduct ion at m/z 257 vs continual DESI-MS analysis time. Each point is a result of sequential 30 s mass spectral scans within a single 10 min exposure of deposited RDX to DESI solvent spray. Error bars are not included because the plot is intended to merely show the trend of decreasing ratio vs constant analysis time.

sample surface for the zero-voltage experiment, other than gravity, is the coaxial flow of carrier gas. At this point, it seems that with the current design, the carrier gas may not be enough to keep a consistent spray flow to the sample surface, resulting in a range of RDX signal response. In terms of monitoring the RDX dimer and monomer signals with changing DESI tip voltage, Figure 7 shows a range of -3.5 to $+3.5$ kV on the spray emitter, with the voltage being generated by an external power supply. Not surprisingly, a positive tip voltage was not efficient for generating negative RDX-based ions in the mass spectra. However, it is interesting to note that the monomer and dimer maxima versus

tip voltage are different and that the monomer is much more dependent on tip voltage, and thus droplet charge, than the dimer. The monomer signal continually increases with increasing negative DESI tip voltage, whereas the dimer is suppressed at higher negative voltages. With more charge available on the desorbed droplets, the competitive ionization processes that benefit dimer formation at lower voltages are not exemplified. The error bars for the monomer signals illustrate that only studying raw signal response may result in poor quantification results. However, the inset of Figure 7 depicts the RDX dimer/monomer ratio with respect to voltage, where the error bars of the ratio of the signals indicate a better means for quantification. The response curve shape is roughly Gaussian about the low-voltage emitter potential, with a peak somewhere between 0 and -500 V within the error bars. This can be explained by the mechanisms reported earlier regarding the total amount of available charge. In the low-voltage case, the smallest amount of charge exists on the declustering desorbed droplets, with the associating RDX dimer units being more proportionately accepting of the charge, owing to their larger cross-sectional areas, and possibly because of the previously proposed mechanisms of dimers more readily incorporating into the periphery of the droplets.^{18,23} Previous work has been reported in which DESI,⁹ and DESSI,³ have been used with zero voltage applied to the emitters. For the DESSI experiment, the drug tamoxifen with a $[M + H]^+$ ion at m/z 372 was actually visible in higher abundance with zero voltage than with 3 kV applied.³ DESI signals have been reported for melittin $[M + 3H]^{3+}$ ions at zero voltage, although these were not considered to be optimum conditions.⁹ In the DESSI experiment, the drug signal molecular ion was greatly enhanced at zero voltage and was attributed to less competition for overall desorbed droplet charge in relation to smaller species present in the droplets.³ It is believed that these observations correlate well with the data and mechanisms presented here for zero-voltage DESI of

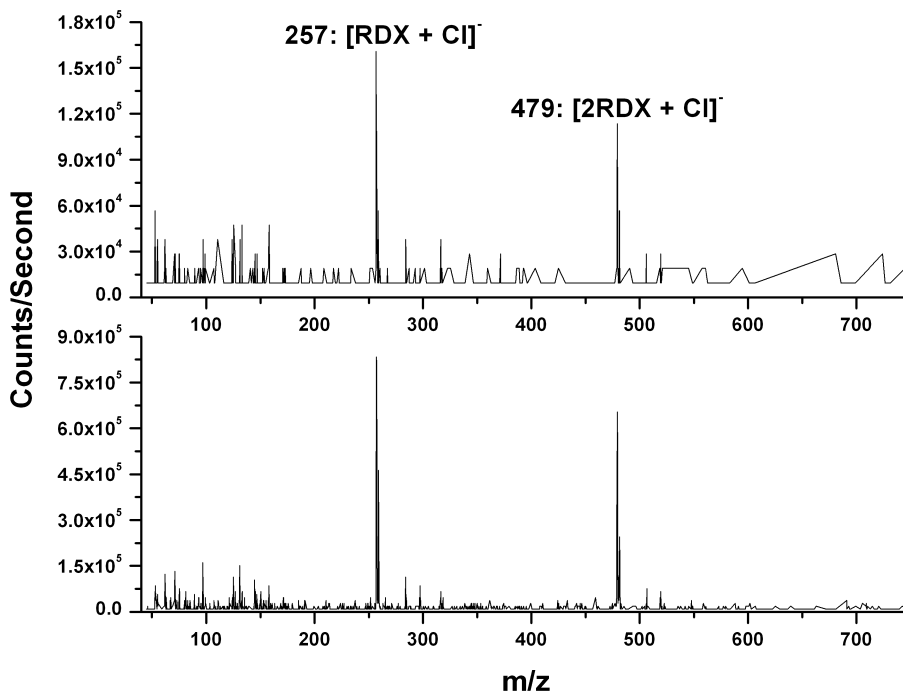


Figure 6. DESI-MS spectra with zero voltage on the spray tip and data acquisition of 60 s. The top spectrum is the minimum detected signal under zero-voltage DESI conditions, and the bottom spectrum is the best expected signals under zero-voltage DESI conditions.

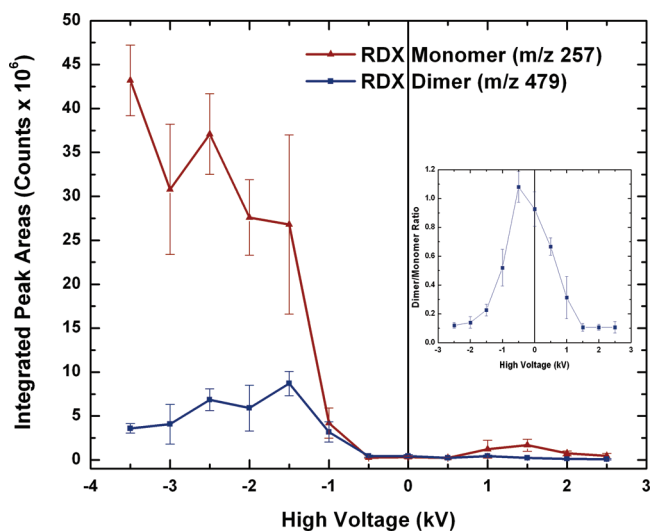


Figure 7. Plot of integral counts for RDX dimer chloride adduct at m/z 479 and RDX monomer chloride adduct at m/z 257 vs DESI tip voltage. Each point is the average of three experiments, with the error bars representing the standard uncertainties at each point. Inset: plot of the ratio of RDX dimer chloride adduct at m/z 479 to RDX monomer chloride adduct at m/z 257 vs DESI tip voltage. Each point is the average of three experiments, with the error bars representing the standard uncertainties at each point.

RDX molecules. On the basis of the DESSI results, it can be extrapolated that the RDX dimer adduct can also be favored over the monomer adduct in zero-voltage conditions because of the apparent increased sensitivity to higher-mass species.³

The use of the RDX dimer chloride adduct to monomer chloride adduct ratios as quantitative measures for the detection of explosives from nanogram to greater than microgram solid amounts can be a viable alternative method to the individual adduct signals that deviate from linearity at high concentrations. The signal responses of the RDX dimer and monomer ions provide

an avenue for increased understanding of the mechanisms involved in the DESI process. By studying the fundamentals of ion formation in DESI, parameters can be appropriately adjusted in order to expand the scope of surface analysis. Additionally, the ion chemistry studied here can be used to tailor the conditions used in IMS experiments, where explosive adducts are often detected after entering the gas phase from solid material.

CONCLUSIONS

The ability to use a DESI ion source with an atmospheric pressure ionization mass spectrometer (API-MS) for explosives analysis can expand the amounts of RDX that can be quantitatively analyzed from nanograms to beyond micrograms of solid deposited material. Since the main RDX adduct integral peak intensities are inconsistent, a method was devised to use the ratio of the RDX dimer to monomer integral signals for determination of the amount of surface RDX present on a sample. The response curve with respect to surface material can be explained through a combination of ESI-like droplet charge effects and solution dynamics in both the DESI droplet pool and the solution-based portions of desorbed droplets, as well as basic chemistry of RDX–RDX intermolecular forces. These mechanisms are also used to explain the mass spectral results of the DESI-MS analysis of RDX versus time and, separately, DESI spray emitter potential. Practical extensions to the possibility of using DESI-MS as a means of explosives detection in the public domain can stem from a ratio of signals being more forgiving to experimental errors because of the raw signal counts being less important. Additionally, the experimentalist can perform a DESI-MS analysis for multiple iterations with different parameter sets if needed for different analytes since the explosive-specific mass spectral peaks can be maintained over long acquisition times. Other benefits of the DESI-MS analysis of explosives include the notion of minimal sample preparation required for surface analysis, the lack of

fragmentation in the mass spectra so that explosive-specific peaks are produced in the highest abundance possible, and the possibility to selectively probe for certain analytes since only what is solvated into the droplet pool will be desorbed into the mass spectrometer. The ability to run the experiment at zero voltage and still obtain explosive-specific mass spectra can also dramatically expand the scope of where and how a DESI-MS analysis can take place. Future work will focus on the ion formation mechanisms of other explosives molecules within explosive mixtures and using the DESI-MS experiment as a scaffold for studying the conditions used in IMS experiments.

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